Original Article

Relationship between the expression of striated preferentially expressed gene (*SPEG*) and the development of atrial fibrillation

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Background: Atrial fibrillation (AF) is a heart rate disorder associated with a high disability rate. A number of genes involved in core coding are also implicated in the development of AF. The striated preferentially expressed gene (*SPEG*) is a newly discovered gene related to the pathogenesis of AF. It promotes disease development by participating in fibrosis and abnormal ion channel function. In this study, the high-risk genes of AF disease were screened by bioinformatics analysis, and the gene polymorphism and gene expression of AF related genes were further analyzed.

Methods: (I) *SPEG* was selected as the core gene of AF according to gene intersection, protein-protein interaction (PPI), and Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses based on Gene Expression Omnibus (GEO) and GeneCards databases; additionally, the diagnostic value of *SPEG* susceptibility was verified in the GSE41177 dataset. (II) The RS576016632 mutation of the *SPEG* gene in patients with AF in Guangxi was detected by unit point sequencing, and SPEG gene expression was detected via quantitative polymerase chain reaction (qPCR).

Results: *SPEG* was identified as a hub gene in the intersecting gene set (*KCNJ4*, *SCN2B*, *MYH7*, *MYL3*, and *ACTA2*). *SPEG* expression was significantly different between the AF and sinus rhythm (SR) groups ($P<0.001$). Additionally, the area under the receiver operating characteristic (ROC) curve was 0.92. AF hub genes were found to be involved primarily in biological processes (BPs) associated with positive regulation of transcription by RNA polymerase II. The G/A mutation at the RS576016632 locus of *SPEG* in patients with AF in Guangxi was identified as a rare mutation.

Conclusions: The RS576016632 locus of *SPEG* in 189 patients was sequenced, but there was no G/A mutation. *SPEG* is closely associated with the development of AF and demonstrates high accuracy in predicting the risk of AF. However, the rs576016632 G/A mutation in *SPEG* may not be a significant risk factor for patients with AF in Guangxi.

Keywords: Striated preferentially expressed gene (*SPEG*); bioinformatics; calcium homeostasis; exon sequencing

Submitted Mar 04, 2025. Accepted for publication Mar 14, 2025. Published online Mar 27, 2025.
doi: 10.21037/jtd-2025-456
View this article at: https://dx.doi.org/10.21037/jtd-2025-456

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Introduction

Atrial fibrillation (AF) is a commonly observed type of rapid atrial arrhythmia in clinical practice, which often results in severe complications such as heart failure and cardiogenic embolism (1). AF can cause changes in cardiac function, leading to hemodynamic disturbances, and the left ventricular ejection fraction in patients with AF is approximately 25% lower than that in healthy individuals. Moreover, patients with AF exhibit reduced atrial filling or increased pressure in both atria, resulting in a higher incidence of arrhythmogenic cardiomyopathy (2-4). AF has also been linked to extracardiac diseases (1). With the advancements made in basic and clinical research on AF over the past few decades, various risk factors have been identified, including smoking, hypertension, obesity, diet, age, and sex. Moreover, genetic risk factors have garnered increased attention (2). However, AF is a complex polygenic condition, and its pathogenesis remains unclear (5).

AF can be categorized into valvular AF (VAF) and nonvalvular AF (NVAF). The pathogenesis of AF involves both the initiation and maintenance of pathogenic mechanisms. Genetic mutations influence the occurrence and progression of AF to a certain extent (6,7). Research has identified the crucial role of ion channels in AF pathogenesis (8). Therefore, genetic mutations that affect ion channel structures have become a focus of research (9). Recently, various genes and factors related to calcium ion channels have been identified. The early release of calcium stored in the sarcoplasmic reticulum may be related to the occurrence and maintenance of AF. In patients with chronic AF, fibrous scars formed by greater fibrosis can also be seen (10). Striated preferentially expressed gene (*SPEG*), a critical regulator of calcium ions, contributes to the occurrence and maintenance of AF by influencing the duration of action potentials (11,12).

AF is the result of multigene synergism. First, a recent study has shown that *SPEG* is involved in the formation of a tripartite structure composed of sarcoplasmic reticulum, transverse striae, and other components, which plays a key role in the transport of calcium ions in cardiomyocytes, actively moving calcium ions into the sarcoplasmic reticulum for storage (4). Second, *SPEG* is also highly expressed in the mitochondria of muscle cells, with the *SPEG* alpha subunit being specifically expressed in cardiomyocytes. Abnormalities, namely single-nucleotide polymorphisms (SNPs) and single-nucleotide variations (SNVs), can also cause mitochondrial ridge structure abnormalities and a decrease in the mitochondrial ATP production rate (13).

In this study, we analyzed the GSE41177 and GSE128188 datasets from the Gene Expression Omnibus (GEO) database and known human pathogenic genes related to AF obtained from the GeneCards database (http://www.genecards.org/) in order to confirm the involvement of *SPEG* in AF pathogenesis. Subsequently, using bioinformatic tools such as RStudio, Cytoscape software, and the Microbioinformatics website (http://www.bioinformatics.com.cn/), we aimed to screen, analyze, and summarize the expression of *SPEG* in the myocardial tissue of individuals with AF and those with sinus rhythm (SR). Additionally, the relationship between *SPEG* and AF was clarified through receiver operating characteristic (ROC) curve analysis. Finally, by screening mutation sites in the Human Genome Project we identified exons harboring AF-associated SNPs. Samples were collected from 129 patients with NVAF, 60 patients with VAF, and 100 individuals with SR in the Guangxi region. Exon sequencing was

Highlight box

Key findings

- The striated preferentially expressed gene (*SPEG*) gene is associated with the incidence of atrial fibrillation, and the common mutation sites of *SPEG* in the local population are different from those in other parts of the world.

What is known and what is new?

- *SPEG* has been identified as a critical hub gene in the pathogenesis of atrial fibrillation. SPEG interacts with sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase 2a (*SERCA2a*) to promote the movement of calcium ions.
- In this study, *SPEG* expression differed between the atrial fibrillation and sinus rhythm groups. Receiver operating characteristic curve analysis indicated that *SPEG* expression had a high accuracy (area under the curve >0.90) in predicting atrial fibrillation. Atrial fibrillation hub genes are involved in positive regulation of transcription by RNA polymerase II. The presence of a rare G/A mutation in *SPEG* at the RS576016632 locus patients with atrial fibrillation from Guangxi differs from the global data of the Human Genome Project.

What is the implication, and what should change now?

- The abnormal expression of *SPEG* was positively correlated with the incidence of atrial fibrillation.
- The expression of *SPEG* varies significantly across individuals as does the pathogenesis of atrial fibrillation associated with it.
- Carefully screening of other loci for the *SPEG* gene among the Guangxi population should be conducted to determine the gene frequency in this region.

Table 1 Information on the RS576016632 mutation site

SNP	Location	Mutation type	Mutation site	Amino acid site
RS576016632	Coding region	Missense mutation	G/A	NP:005867.3:p.Arg5Leu

To determine the specific location and obtain details of the *SPEG* mutations at the RS576016632 site, we analyzed available information on the UniProt and NCBI websites. SNP, single-nucleotide polymorphism; *SPEG*, striated preferentially expressed gene; NCBI, National Center for Biotechnology Information.

then conducted to investigate the mutation status of the RS576016632 locus of *SPEG* in the NVAF, VAF, and SR groups. We present this article in accordance with the STREGA reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-2025-456/rc).

Methods

Study population and data acquisition

Using the search term "atrial fibrillation", we retrieved two datasets, GSE41177 and GSE128188, from the GEO database (www.ncbi.nlm.nih.gov/geo). The GSE41177 dataset consisted of gene expression profile data from 32 atrial tissue samples in the AF group and six atrial tissue samples in the SR group. Meanwhile, the GSE128188 dataset comprised gene expression profile data from 10 atrial tissue samples in the AF group and four atrial tissue samples in the SR group. Detailed information about these datasets is presented in *Table 1*.

Differential gene expression analysis

Gene expression matrices of the GSE41177 and GSE128188 datasets were downloaded and subjected to differential gene expression analysis using the "GEOquery" and "Limma" packages in the R version 4.2.2 software (RStudio). In the GSE41177 dataset, differentially expressed genes (DEGs) were identified using a threshold of P value <0.05 and $|\log_2$ fold change$|$ >0.50. In the GSE128188 dataset, DEGs were identified using a threshold of P<0.05 and $|\log_2$ fold change$|$ >0.40. Subsequently, differential gene expression data were exported and visualized as volcano plots and heatmaps using the Microbioinformatics online website, with the target genes being highlighted.

Identification of potential AF-related target genes

Known AF-related human genes were identified and downloaded from the GeneCards database using the term "atrial fibrillation". Genes with a relevance score of >2 in each dataset were selected as the AF-related genes. Subsequently, Venn diagrams of the DEGs from the GSE41177 and GSE128188 datasets intersecting with the genes obtained from the GeneCards database were plotted. The overlapping genes were considered potential AF target genes and further analyzed.

Protein-protein interaction (PPI) analysis of the potential target genes and identification of hub genes

To evaluate protein interactions among the potential target genes, we employed the Search Tool for the Retrieval of Interacting Genes (STRING; version 12.0; https://cn.string-db.org/). First, potential target genes were imported into STRING to construct a PPI network. The confidence threshold (medium confidence) was set to 0.15. Subsequently, the corresponding results were imported into the Cytoscape software (version 3.10.0) for hub gene screening and visualization. Through the CytoNCA plugin in Cytoscape, betweenness centrality (BC), closeness centrality, and degree centrality were selected as the main criteria, with BC being the primary selection standard. The top 50 genes under these three criteria were identified as hub genes.

Functional enrichment analysis of hub genes

Gene Ontology (GO) is a framework for the functional annotation of genes, categorizing them into biological process (BP), cellular components (CC), and molecular function (MF). Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis is the most common approach employed for pathway annotation. In this study, hub genes were imported into the Database for Annotation, Visualization and Integrated Discovery (DAVID) online database (version 6.8; https://david.ncifcrf.gov/summary.jsp) for analysis. The top 10 entries with P<0.05 were selected for visualization via the Microbioinformatics website.

Analysis of SPEG expression

The expression level of *SPEG* in both the AF and SR groups was analyzed using the validation dataset GSE41177, and the results were visualized using the "ggpubr" package in RStudio. In addition, the Human Protein Atlas online database (http://www.proteinatlas.org/) was used to analyze the expression level of *SPEG* in various tissues. This database provides information on the expression of protein-coding genes in all major human tissues and organs.

Analysis of the diagnostic value of SPEG

The GSE41177 dataset was used to validate expression level*s* of *SPEG*. After the expression data for each sample were downloaded, the software (IBM Corp., Armonk, NY, USA) was employed to perform ROC curve analysis, which enabled determination of the specificity, positive predictive value, sensitivity, likelihood ratio, and negative predictive value of *SPEG* at all possible thresholds. Subsequently, the accuracy of *SPEG* in predicting AF susceptibility was evaluated based on the results of ROC curve analysis.

Selection and sequencing of target gene loci

Next, the *SPEG* gene loci were searched in the SNP database on the PubMed website (https://www.ncbi.nlm.nih.gov/snp/). The criteria for SNP site selection were as follows: (I) sites located in functional regions of the gene, such as the promoter, coding, and exon regions; (II) sites with a minor allele frequency of ≥2%; and (III) sites reported to be associated with AF susceptibility in the literature. Based on these criteria, the RS576016632 locus was identified.

We selected 129 patients with NVAF and 60 patients with VAF who had been admitted to the Department of Cardiovascular Medicine and Cardiothoracic Surgery at the Affiliated Hospital of Youjiang Medical University for Nationalities and Wuzhou People's Hospital from November 2021 to February 2024 to form the case group. In addition, 100 individuals diagnosed with SR at the Physical Examination Center of both hospitals in the same period were selected as the control group. The general clinical data of patients age, creatine kinase MB isoenzyme (CK-MB), partial thromboplastin time (PTT), international normalized ratio (INR), activated partial thromboplastin time (APTT), creatinine (CREA), left atrial end-systolic transverse diameter (LAsD), right atrial end-systolic transverse diameter (RAsD), ejection fraction (EF), sex, cardiac troponin I (cTnI), myoglobin (MYO), and platelet (PLT) were collected for statistical analysis. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study received approval from the Ethics Committees of the Affiliated Hospital of Youjiang Medical University for Nationalities (No. YYFY-LL-2023-173) and Wuzhou People's Hospital (No. 2022093). All participants and their families provided written informed consent.

Experimental and sequencing methods

Morning fasting venous blood samples (4 mL) were collected from the study participants into anticoagulant tubes containing EDTA K2. After labeling, all samples were stored at −80 ℃ until analysis. Genomic DNA was then extracted using a magnetic bacterium genomic DNA kit (Xi'an Biolab Biotechnology Co., Ltd., Xi'an, China). After extraction, the quantity and quality of DNA were determined using agarose electrophoresis. The samples were then stored at −80 ℃ until sequencing. Subsequently, the extracted DNA was sent to the Wuhan Branch Office of Beijing Genomics Institute for locus DNA sequencing of *SPEG* via the Sanger sequencing method to identify mutations in the corresponding samples. The primer sequences used for the amplification and sequencing are shown in Table S1. We randomly selected 10 samples from the AF group and the control group and sent them to Beijing Genomics Institution (Wuhan, China) for quantitative polymerase chain reaction (qPCR) detection. We also analyzed *SPEG* gene expression in the samples of the two groups.

Statistical analysis

All statistical analyses were performed using the SPSS 19.0 software package (IBM Corp.). The clinical data were all in line with the normal distribution detected by SPSS 19.0. Measurement data were further tested by the independent samples *t*-test for specificity, and count data were tested via Chi-squared tests. The results of gene expression analysis of the GSE41177 and GSE128188 datasets were consistent with positive distribution and were further analyzed via the *t*-test. Cluster analysis of BP, MF, and CC terms was performed, and the hypergeometric P value was calculated. The Chi-squared test was used for determining Hardy-Weinberg equilibrium of count data and genotype. Results

with a P<0.05 were considered statistically significant.

Results

Volcano plot and heatmaps of DEGs

In this study, we identified a total of 19,540 DEGs between the AF and SR control groups in the GSE41177 dataset. Of these, 19,531 genes were upregulated, and 9 genes were downregulated in the AF group. A volcano plot of differential gene expression is shown in *Figure 1A*, and a heatmap of the top 50 DEGs is displayed in *Figure 1B*. Meanwhile, there were 2,258 DEGs in the GSE128188 dataset between the AF and SR groups, of which 918 were upregulated and 1,340 were downregulated. A volcano plot of the differential gene expression profile is shown in *Figure 1C*, and a heatmap of the top 50 DEGs is shown in *Figure 1D*.

Screening of potential target genes

Screening of the GeneCards database revealed 5,076 genes associated with AF, of which 2,193 genes had a relevance score of >2. Notably, a total of 100 DEGs were shared across the GSE41177, GSE128188, and GeneCards datasets. These were selected for further analysis. Among the 100 overlapping genes, *SPEG* was identified as a potential target (*Figure 2*).

PPI network analysis and hub gene screening

The 100 intersecting genes were imported into the STRING database for PPI network analysis. After the removal of isolated nodes, the constructed PPI network included 95 nodes (proteins) and 1,165 edges (PPIs), with an average node degree of 24.5 and a PPI enrichment P value of <0.001. Subsequently, the corresponding .TSV file was exported and imported into Cytoscape software. Topological calculations were performed with the CytoNCA plugin, and the top 60 genes ranked by BC were considered hub genes. The hub genes included *MYH7*, *JUN*, *VWF*, *MYL3*, *NPPA*, *CACNB2*, *KCNE4*, *SYNE2*, *SPEG*, and *KCNAB2* (*Figure 3*).

GO and KEGG enrichment analyses

KEGG pathway enrichment results are presented as a bubble chart in *Figure 4A*. Hub genes were primarily enriched in the cAMP signaling, cardiac muscle contraction, cyclic guanosine monophosphate-protein kinase G (cGMP-PKG) signaling, calcium signaling, and apelin signaling pathways. The GO enrichment analysis results, including BP, CC, and MF terms, are displayed in bar charts. As shown in *Figure 4B*, the hub genes were predominantly associated with the following BPs: cell adhesion, regulation of blood pressure, calcium-mediated signaling, cell-cell signaling, positive regulation of heart rate, and positive regulation of ATP-dependent activity.

With regard to CC terms, hub genes were primarily enriched in the synapse, protein-containing complex, dendrite, cell surface, axon, sarcolemma, presynaptic membrane, actin cytoskeleton, myosin II complex, and sarcomere.

Regarding MF terms, hub genes were primarily enriched in calcium ion binding, heparin binding, actin binding, hormone activity, actin filament binding, collagen binding, voltage-gated calcium channel activity involved SA node cell action potential, myosin II heavy chain binding, microfibril binding, and high voltage-gated calcium channel activity.

SPEG expression in various tissues

The GSE41177 dataset, comprising 32 AF samples and 6 SR samples, was used as the validation dataset. Notably, the AF group exhibited significantly higher *SPEG* expression than did the SR group (P<0.001), as shown in *Figure 4C*.

ROC curve analysis of SPEG expression

Using the GSE41177 dataset, ROC curve analysis was performed to evaluate the accuracy of *SPEG* in predicting AF susceptibility. *SPEG* expression was significantly higher in the AF group than in the SR group in the validation dataset (P<0.001). Additionally, the area under the ROC curve (AUC) for *SPEG* expression was 0.92, indicative of high accuracy in predicting AF risk (*Figure 4D*).

Patient data

By comparing the clinical data of patients with the RS576016632 mutation in the AF group to that of patients without the mutation, we found statistically significant differences in age, PTT, INR, APTT, LAsD, RAsD, EF (P<0.001), CREA (P=0.03) and CK-MB (P=0.006). There was no significant difference in gender (P=0.35), cTnI (P=0.24), MYO (P=0.09) and PLT (P=0.07) (*Table 2*).

The RS576016632 locus is situated within the coding region of *SPEG* (*Table 1*). This mutation is a G>A

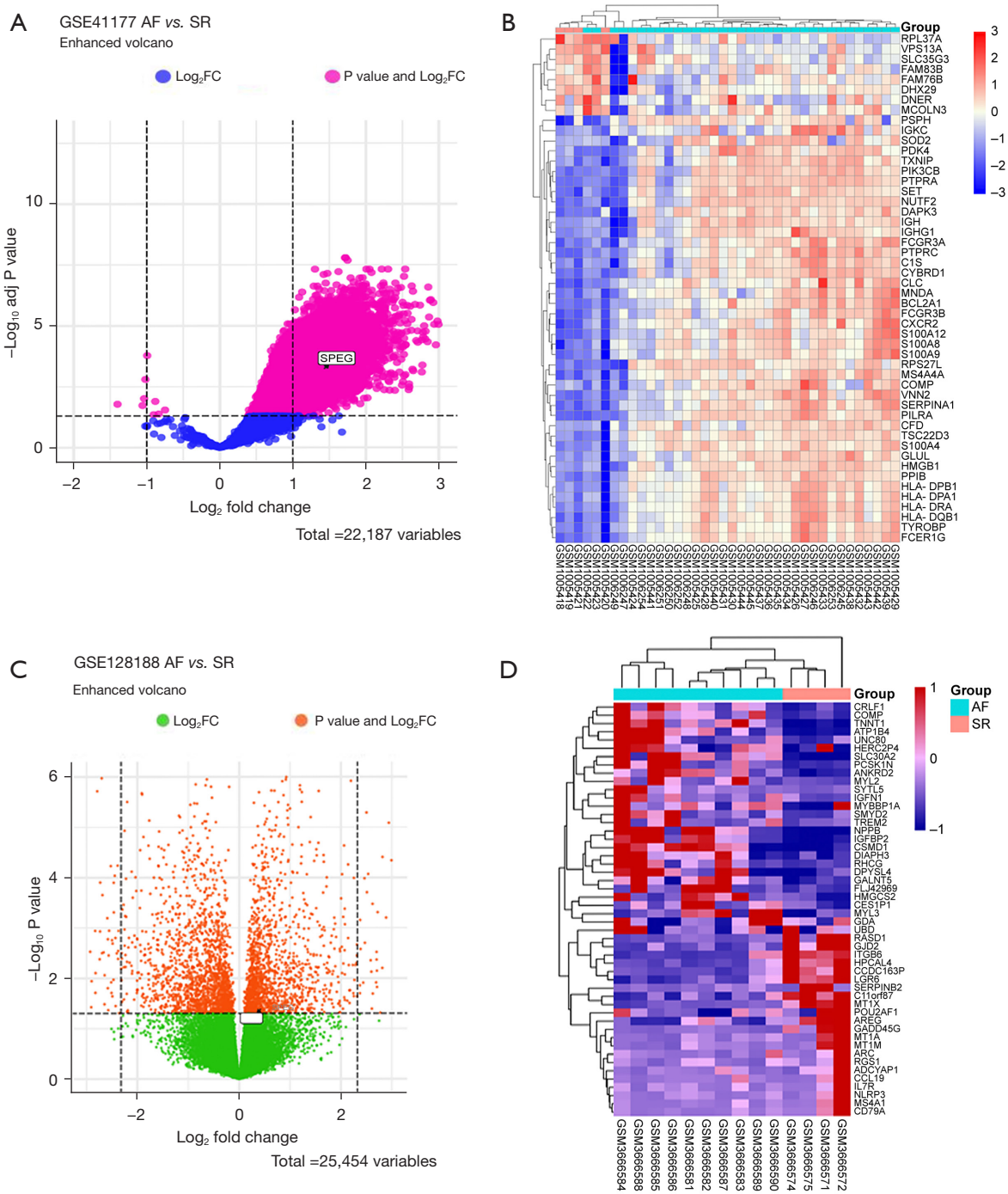

Figure 1 Volcano plots and heatmaps for the AF and SR groups. (A) Volcano plot of the differential gene expression profile. (B) Heatmap of the top 50 DEGs. (C) Volcano plot of differential gene expression in the GSE128188 dataset, which contained a total of 2,258 DEGs between the AF and SR groups, with 918 genes with upregulated expression and 1,340 genes with downregulated expression in the AF group. (D) Heatmap of the top 50 DEGs. AF, atrial fibrillation; SR, sinus rhythm; FC, fold change; DEG, differentially expressed gene.

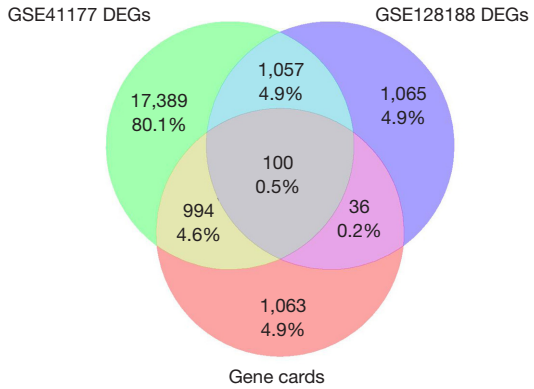

Figure 2 Venn diagram of DEGs in each dataset. There were 100 shared genes among the three datasets. Among these 100 common genes, *SPEG* was identified as a potential target. DEG, differentially expressed gene; *SPEG*, striated preferentially expressed gene.

substitution, resulting in a change of arginine with leucine in the protein product, causing a substantial alteration in protein structure. To determine the specific location and obtain details on the rs576016632 variant, the UniProt (https://www.uniprot.org) and National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/gap/phegeni) databases were used (*Table 3*). The genotype distribution of rs576016632 was examined in 129 patients with NVAF, 60 patients with VAF, and 100 control individuals from the Guangxi region. The rs576016632 *SPEG* locus was sequenced in 189 patients with AF and 100 controls with SR without the mutation. The results of statistical analysis were significantly different compared to those of the Human Genome Project (P=0.049).

Genotypes of the rs576016632 locus in 189 samples sequenced in the study region's population were compared

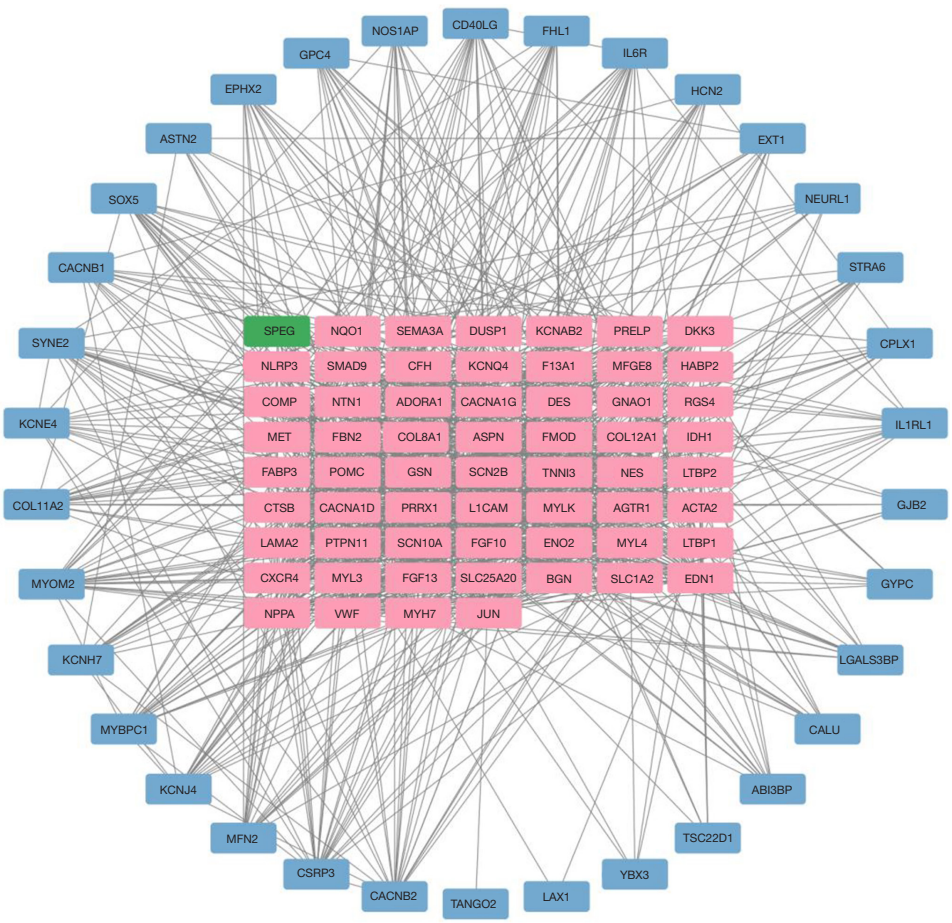

Figure 3 Protein-protein interaction diagram. The protein-protein interaction network shows the top 50 genes, based on BC, which were selected as the hub genes. These hub genes included *MYH7*, *JUN*, *VWF*, *MYL3*, *NPPA*, *CACNB2*, *KCNE4*, *SYNE2*, *SPEG*, and *KCNAB2*. BC, betweenness centrality; *SPEG*, striated preferentially expressed gene.

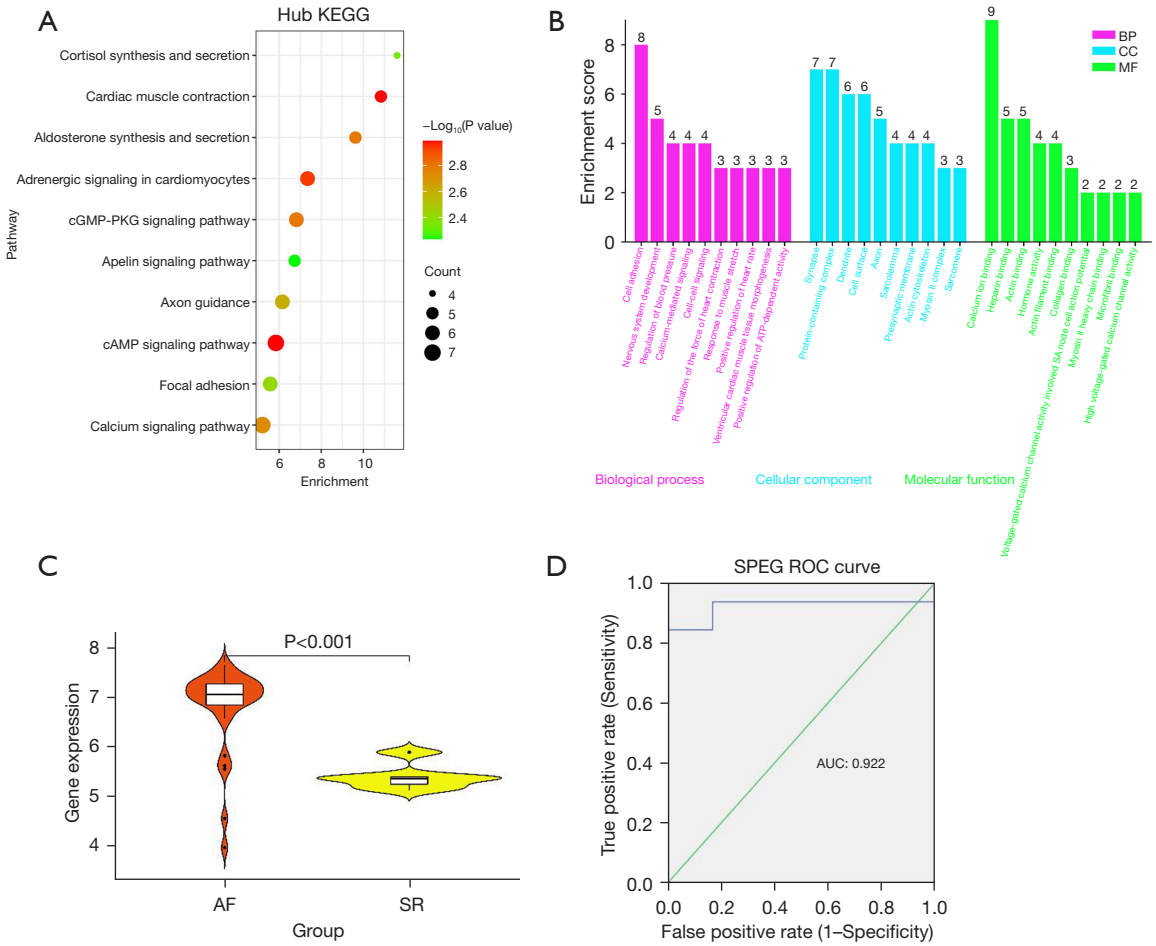

Figure 4 Enrichment analysis chart and ROC curve graph. (A) Bubble chart of the KEGG pathway enrichment results. (B) Bar charts of GO enrichment analysis results, including for BP, CC, and MF. (C) SPEG expression was significantly higher in the AF group than in the SR group (P<0.001). (D) Analysis of the GSE41177 dataset using ROC curve analysis to evaluate the accuracy of *SPEG* in predicting AF risk. KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological process; CC, cellular component; MF, molecular fraction; AF, atrial fibrillation; SR, sinus rhythm; *SPEG*, striated preferentially expressed gene; ROC, receiver operating characteristic; AUC, area under the curve; GO, Gene Ontology; cGMP-PKG, cyclic guanosine monophosphate-protein kinase G; cAMP, cyclic adenosine monophosphate.

with single-nucleotide distributions in the global population and populations from South Africa, the United States, Spain, Europe, and Latin America.

In order to assess *SPEG* gene expression in the control group and the AF group, we selected 10 samples from each group for qPCR. Compared with the control group, *SPEG* messenger RNA (mRNA) expression was significantly decreased in the AF group (*Figure 5*).

Discussion

In this study, we found that *SPEG* expression was upregulated in AF through bioinformatic analysis. Additionally, we demonstrated that *SPEG* could accurately predict the risk of AF. Moreover, *SPEG* was found to correlate with ion channel function. AF is considered to be a complex condition marked by impairments in the electrophysiological activity of the heart and is caused by various factors. Pathological changes, such as abnormal ion channel function and myocardial fibrosis, can promote and maintain the onset of AF, with common calcium, potassium, and sodium pumps commonly being affected (14,15). The current treatment strategies focus on controlling the ventricular rate or restoring AF to SR. However, owing

to the high recurrence rate of AF and the adverse effects of these treatments, investigations into new therapeutic approaches are underway. There has been a recent shift in research toward targeted therapies, including pathway- and substrate-specific agents (upstream therapies) (16,17).

Table 2 Clinical data results

Variables	$t/\chi^{2\dagger}$	P value
Age	7.927	<0.001
Sex (male/female)	0.865	0.35
cTnI (μg/mL)	−1.192	0.24
MYO (μg/mL)	−1.706	0.09
CK-MB (U/L)	−2.754	0.006
PTT (s)	7.092	<0.001
INR	7.560	<0.001
APTT (s)	4.548	<0.001
CREA (μmol/L)	2.201	0.03
PLT ($\times10^9$)	−1.795	0.07
Echocardiography		
LAsD (mm)	15.285	<0.001
RAsD (mm)	9.388	<0.001
EF (%)	−4.287	<0.001

†, age and sex are χ^2 values, and the others are *t* values. cTnI, cardiac troponin I; MYO, myoglobin; CK-MB, creatine kinase MB isoenzyme; PTT, partial thromboplastin time; INR, international normalized ratio; APTT, activated partial thromboplastin time; CREA, creatinine; PLT, platelet; LAsD, left atrial end-systolic transverse diameter; RAsD, right atrial end-systolic transverse diameter; EF, ejection fraction.

Abnormal calcium concentration in the sarcoplasmic reticulum and muscular duct system is closely related to the onset of AF due to abnormal coding of calcium ion channels (18). Mutations in genes encoding ion channels and abnormalities in related accessory proteins and factors contribute to the emergence and persistence of AF (19,20). Determining the molecular mechanisms involved in the pathogenesis of AF has facilitated the development of effective treatments. In recent years, multiple risk factors for AF have been identified, including a growing number of genetic mutations and polymorphisms (21).

In this study, 72 AF-related genes were identified from the GEO and the GeneCards databases through bioinformatic analysis, and 50 core genes were selected based on node centrality via a protein interaction website. GO and KEGG enrichment analyses revealed that calcium channels play a key role in AF pathogenesis, which is consistent with the findings of a previous study (6). We subsequently examined the relationship between the key *SPEG* gene and AF development in relation to calcium homeostasis. The RS576016632 G/A mutation locus was screened using the NCBI database to assess the risk of AF in patients living in Guangxi.

In recent years, various bioinformatic methods have emerged, but only a few have been widely adopted in China, which may be attributed to the limitations in the capabilities of existing methods to appropriately account for significant ethnic diversity, disease specificity, and differences in living habits across patient populations. As common bioinformatic tools, such as Regmex, require further validation in this population, we did not use Regmex or other methods for

Table 3 rs576016632 mutation distribution in various regions

Region	No. of cases	Allele, n (%)			
		G	A	T	C
Guangxi	189	378 (100.00)	0	0	0
World	52,087	104,157 (99.98)	1 (<0.001)	15 (<0.001)	1 (<0.001)
South Africa	661	1,322 (100.00)	0	0	0
United States	346	691 (99.99)	1 (0.01)	0	0
Spain	504	1,008 (100.00)	0	0	0
Europe	7,698	15,395 (100.00)	0	0	1 (<0.001)
Latin America	10,700	21,385 (99.99)	0	15 (<0.001)	0
P value	0.049				

G, guanine; A, adenine; T, thymine; C, cytosine.

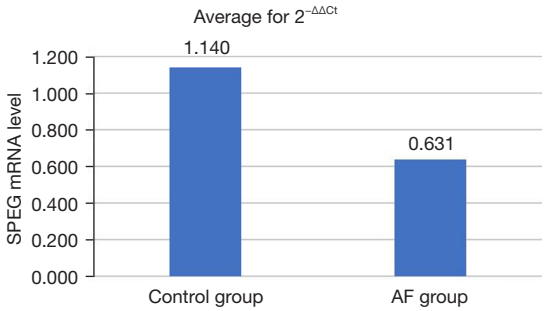

Figure 5 The expression of *SPEG* mRNA in the AF group was lower than that in the control group. The figure shows the qPCR results of 20 cases. *SPEG*, striated preferentially expressed gene; AF, atrial fibrillation; qPCR, quantitative polymerase chain reaction.

identifying important motifs in sequence lists, nor did we conduct motif analysis or traditional k-mer screening. We screened the local population by sequencing exon loci with high mutation frequency in the Human Genome Project.

Genome-wide association studies have identified several AF susceptibility-related mutation loci, specifically in genes associated with calcium channels, and genes upstream and downstream of *SPEG* have been examined to determine whether there is a synergistic effect. *SYNPO2L8* and *TTN* are involved in atrial myocardial remodeling, which leads to AF in cardiomyopathy (22). Mutations in *SPEG* (linked to myocyte diseases) that are associated with dilated cardiomyopathy and cardiomyopathy-induced AF have been identified (22,23). Moreover, *TTNtv* has been found to be significantly enriched in familial and early-onset AF. In one study, the genetic risk in the population of British residents of European descent was assessed based on predicted loss of function variants and polygenic risk (24). However, there was no obvious familial clustering of AF in the population within the study area. Our study did not involve family-based research or multimethod susceptibility assessments. Instead, we focused on the role *SPEG* plays in the electromagnetic homeostasis related to AF pathogenesis and examined the rs576016632 G/A mutation in patients with AF.

The *SPEG* alpha subunit is overexpressed in cardiomyocytes, promoting calcium channel maturation via ridge development and phosphorylation. Abnormal expression of the *SPEG* gene causes mitochondrial dysfunction, characterized by significantly reduced ATP production and a marked decrease in the concentration of calcium ions in the sarcoplasmic reticulum. An unstable calcium concentration is closely related to electrical activity in AF. Factors upstream and downstream of *SPEG*, such as RyR2, also play a critical role in the development of AF, contributing to a decline in sarcoplasmic reticulum function through phosphoric acid deficiency (10). Conversely, mutations in this gene or reduction in its expression can lead to an abnormal increase in calcium ion release, causing delayed afterdepolarizations (DADs) with increased amplitude, which upon reaching the threshold, can trigger AF. The generation of early afterdepolarizations (EADs) and DADs results in shortened action potentials. In addition, phosphorylation of the *SERCA2a* receptor at site T484 increases calcium uptake. Notably, abnormal enhancement of *SERCA2a* receptor activity can accelerate Ca^{2+} cycling, leading to faster production of Ca^{2+} transients and increased activation of sodium-calcium exchangers. This results in membrane depolarization and a reduced DAD threshold, thereby increasing the likelihood of AF development. Stabilizing the junctional membrane complex and ensuring the structural integrity of the *JPH-2* protein is essential for effective electrical conduction. Structural disruption can lead to electrical disturbances, promoting the occurrence and maintenance of AF (6,11,17). Meanwhile, animal experiments have revealed that greater cardiac interstitial fibrosis with abnormal *SPEG* expression. The relationship between fibrosis, membrane potential, and action potential requires further study (13). The rs57601662 *SPEG* variant is a missense mutation, located in the coding region at Chr2:19434991. This mutation, which affects the protein backbone, is a substitution of G with A, resulting in a change of arginine to leucine (5). Ultimately, this alteration changes *SPEG* protein structure, thereby affecting cellular functions and potentially causing AF. In this study, the G/A mutation at this locus found not to be a significant factor in AF development. Bioinformatic analysis of screen data from Taiwan and London revealed a close relationship between *SPEG* expression and AF development. The lack of G/A mutations in the Guangxi region may be attributed to geographic factors. This region is located in southwestern China, bordering several Southeast Asian countries. It is characterized by a subtropical monsoon climate and comprises various ethnic groups, including Han, Zhuang, Bouyei, Miao, Yao, and Shui. The long-term economic underdevelopment and low population mobility within this region make its genetic pool relatively stable and representative. We sequenced the loci of the target gene

SPEG. Target sequences are often regulated by cis-acting and trans-acting elements in noncoding sequences, which can activate, inhibit, or silence gene expression (25). The results in our study may be attributed to the suppression of the promoters or transcription factors upstream of the mutation sites, resulting in downregulated expression.

As one of the common arrhythmia diseases, there are many biomarkers for atrial fibrillation. In this study, the author tried to use *SPEG* gene as one of the biomarkers for atrial fibrillation. However, the specific role of *SPEG* gene still needs further study. In this paper, we analyzed the common biomarkers of atrial fibrillation. By comparing a current study, more than dozens of clear markers of atrial fibrillation have been identified (e.g., leptin, adiponectin, ceramide, ferritin, fibrinogen, interleukin-18, thromboreactive protein-1, acylcarnitine, plasminogen activator inhibitor-1, triglycerides, high-density lipoprotein cholestasis, intestinal microbiome, hyperuricemia, and homocysteine) (26). However, at present, there is no direct interaction between the above markers and the *SPEG* gene studied in this paper. In order to clarify the role of SPEG as a biomarker for atrial fibrillation, the author of this paper will further study the mechanism of *SPEG* gene action.

Finally, data mining and analysis revealed a correlation between *SPEG* expression and AF development. The GEO data indicated that *SPEG* expression is upregulated in patients with AF as compared with that in individuals with SR. In addition, statistical analysis revealed *SPEG* expression in the heart to be a highly accurate predictor of AF risk, indicating a close association between *SPEG* expression and AF pathogenesis. Subsequently, the rs576016632 locus was screened to further clarify the association of *SPEG* with AF. Although our results indicated a negative association between the rs576016632 variant and AF, this finding does not necessarily rule out a correlation between *SPEG* expression and AF pathogenesis as our study involved a small sample, suboptimal sample selection, and incomplete evaluation of mutation sites. Overall, the findings of this study may not be fully representative, and future studies with larger samples are required to investigate other notable loci to further validate the relationship between *SPEG* and NVAF.

This study involved other limitations. Specifically, our investigation relied solely on bioinformatic approaches, and we did not conduct corresponding validation experiments *in vitro* or *in vivo*. Therefore, further research should focus on validation experiments to confirm the conclusions of this study.

Conclusions

SPEG is critically involved in maintaining the stability of electrical conduction mechanisms and calcium homeostasis. The failure to effectively promote the maturation of calcium ion channels leads to abnormal calcium ion concentration in the sarcoplasmic reticulum, which is involved in the formation and persistence of AF (11,27). Furthermore, our bioinformatic analysis demonstrated that the hub genes associated with AF may play key roles in the development of AF through various pathways and processes, including cardiac conduction regulation of heart rate, calcium ion transmembrane transport, voltage-gated calcium channel complex formation, MAPK signaling, cAMP signaling, and calcium signaling. These findings align with those of previous studies (15,28-30). Moreover, rs576016632 variant was not detected in this study, and *SPEG* gene expression in the AF group was lower than that in SR group, but the difference between the two was not statistically significant. Our results might have been biased by the small sample size and population specifics in the geographic region, and thus further research is needed.

Acknowledgments

We would like to thank Editage (www.editage.cn) for the English language editing.

Footnote

Reporting Checklist: The authors have completed the STREGA reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-2025-456/rc

Data Sharing Statement: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-2025-456/dss

Peer Review File: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-2025-456/prf

Funding: This work was supported by the Project of Improving the Basic Scientific Research Ability of Young College Teachers in Guangxi (No. 2023KY2019 to B.D.) and the 2022 Wuzhou City Health Commission Scientific Research Project (No. WZWS-F2022012 to B.D.). Both funds supported genetic sequencing.

Conflicts of Interest: All authors have completed the ICMJE

uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-2025-456/coif). B.D. reports funding, the provision of study materials, medical writing, and support for article processing charges from the Project of Improving the Basic Scientific Research Ability of Young College Teachers in Guangxi (No. 2023KY2019), and the 2022 Wuzhou City Health Commission Scientific Research Project (No. WZWS-F2022012). Both funds supported genetic sequencing. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study received approval from the Ethics Committees of the Affiliated Hospital of Youjiang Medical University for Nationalities (No. YYFY-LL-2023-173) and Wuzhou People's Hospital (No. 2022093). All participants and their families provided written informed consent.

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(English Language Editor: J. Gray)

Cite this article as: Wei J, Yu L, Liu Y, Ye S, Song B, Deng Y, Lu Q, Deng B. Relationship between the expression of striated preferentially expressed gene (*SPEG*) and the development of atrial fibrillation. J Thorac Dis 2025;17(3):1723-1735. doi: 10.21037/jtd-2025-456